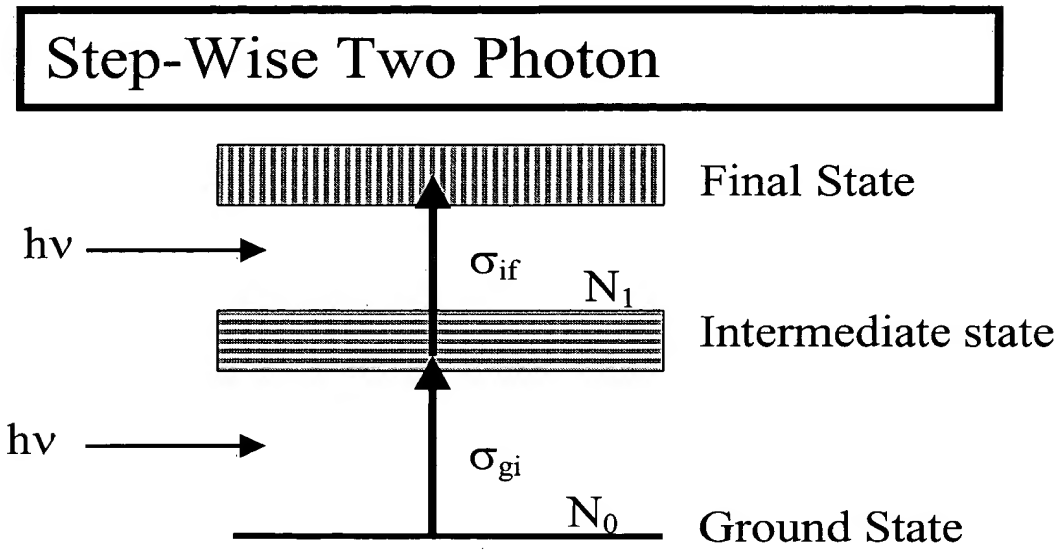
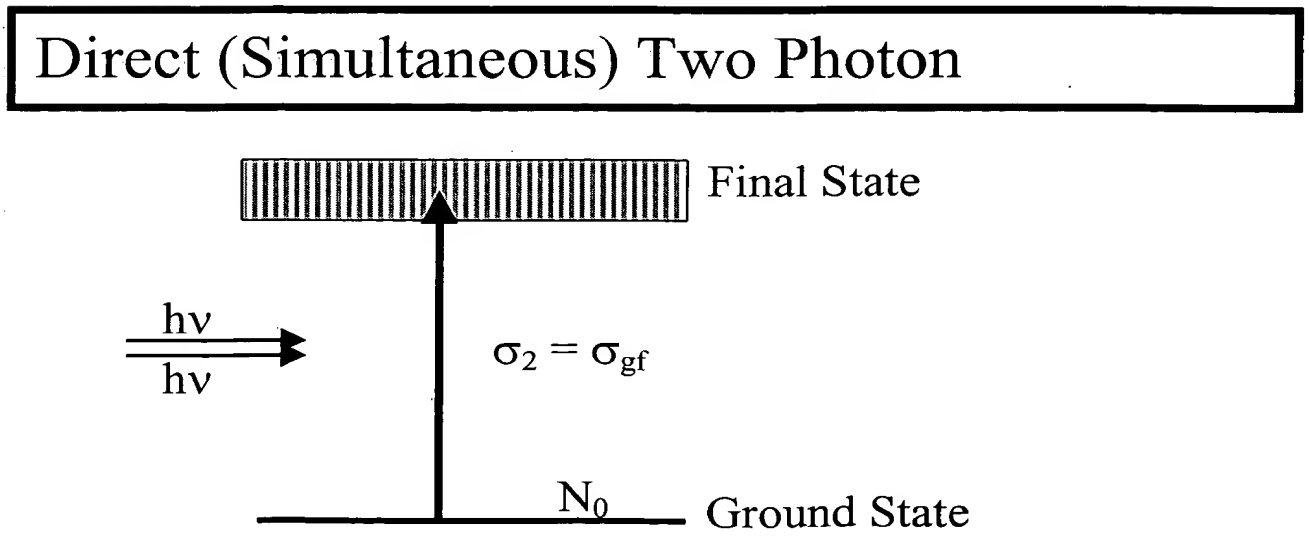
The attached Declaration of George Ian Allan Stegeman under 37 C.F.R. § 1.132 ("Stegeman Declaration") is submitted to show that the present application teaches the use of simultaneous 2-photon excitation.

The simultaneous two-photon excitation phenomena (commonly known as Two Photon Absorption) and the step-wise two-photon excitation phenomena (commonly known as Excite State Absorption or Reversible Saturated Absorption) are shown in the following figure:





(Stegeman Declaration ¶5). As shown in these figures, the use of simultaneous two-photon absorption involves the simultaneous absorption of two photons (photon energy hc/λ) of a given wavelength λ which leads to the excitation of a two-photon active absorption state at the wavelength $\lambda/2$, i.e. eneregy $2hc/\lambda$ above ground state (Id.). Thus, the wavelength of the exciting radiation is specified for a given material and, for optimum results, the material should be as transparent as possible at the exciting wavelength (Id.). On the other hand, as shown in the above figures, in stepwise or sequential two-photon absorption, two sequential absorption events occur, sequentially in time, each involving one photon (photon energy $hc\lambda$) (Id.). The absorption of the first photon requires significant single photon absorption activity into an intermediate state (Id.). This state is evidenced by a lack of transparency at that wavelength (Id.). A second, single photon absorption from that first intermediate state into the final state occurs near or at the peak of the second one photon absorption (final) state (Id.). The desirability of good transparency at the exciting wavelength range (i.e. 700 – 1300 nm) for photodynamic therapy is discussed on page 8, line 10 to page 9, line 5 of the present application (Id.). Since, as noted above, transparency is important for simultaneous two-photon absorption, and absorption is important for step-wise two-photon absorption, one would recognize that the present application intends to use simultaneous two-photon absorption in carrying out photodynamic therapy (Id.).

The sentence bridging pages 1 and 2 of the present application states:

> To date, two major technical approaches have been used to achieve frequency upconversion lasing: one is based on direct two-photon (or multi-photon excitation of a gain medium (two-photon pumped); the other is based on sequential stepwise multi-photon excitation (stepwise multi-photon pumped).

(Stegeman Declaration ¶ 6). With the latter of these 2 technical approaches clearly referring to sequential multi-photon excitation, as discussed above, it is apparent that the former alternative is describing simultaneous multi-photon excitation (Id.).

In describing the two-photon absorption or excitation phenomena utilized by applicants, the present application sets forth, at page 51, line 6, the following formula:

$$I(L)=I_o/(1 + I_oL\beta)$$

(Stegeman Declaration ¶ 7). The present application proceeds to describe this equation as follows on page 51, lines 7-10:

> I(L) is the transmitted incident intensity; L is the thickness of the matrix material; I_o is the incident infrared intensity; and ß is the TPA coefficient of the sample medium and is a linear function of the concentration of the styryl compound in the matrix.

(Id.). See also page 97, line 30 to page 98, line 11 of the present application (Id.). This formula is well known by scientists in this area to refer to simultaneous two-photon excitation with β being defined in the field of nonlinear optics as the simultaneous two-photon absorption coefficient (Id.). For step-wise two-photon absorption, the relationship is not given in terms of the intensity or irradiance, but in terms of an integral over the pulse energy, i.e. the energy or fluence F (Id.). On page 98, line 3, β is defined directly in terms of the molecular two-photon absorption coefficient σ_2 as follows:

$$\beta=\sigma_2 N_o= \sigma_2 N_o d_o \times 10^{-3}$$

(Id.). N_o is the molecular density of the dopant, σ_2 is the molecular TPA coefficient of the same dopant, and d_o is the concentration of dopant (Id.). This is the formula for simultaneous two-photon absorption but not for the step-wise process which contains the product of the two single photon cross-section coefficients σ_{gi} and σ_{if}, as indicated in the above figures -- one for the transition from the molecular ground state to the first excited state (i.e. the intermediate state) and one for the transition from the first excited state to the second excited state (i.e. the final state) (Id.). Furthermore, the formula for step-wise two-photon excitation would also require values for the pulse duration of the laser and the relaxation time of the first (intermediate) excited state (Id.). Thus, the above formulae in the present application would indicate to one of ordinary skill in the art that a simultaneous two-photon excitation process is being used (Id.).

The experimental data set forth in the '625 application demonstrates that the applicants were utilizing simultaneous two-photon excitation (Stegeman Declaration ¶8).

Figure 7 of the present application shows the absorbance spectrogram of a "dye1" doped film used in accordance with the present application, where this dye has no absorbance when subjected to incident photons with a wavelength greater than 600 nm, most specifically the wavelength of the Nd:YAG laser at 1060 nm (Id.). This dye and its method of preparation are described on page 95 of the present application in Example 6 (Id.). Figure 9, as described in Example 11 (pages 97-98 of the present application), shows that when dye1 (i.e. trans-4[p-(N-ethyl-N-hydroxyethyl amino)styryl]-N-methylpyridinium tetraphenyborate) is subjected to a Nd:YAG laser of wavelength of 1060 nm (i.e. 1.06 μm), an intensity-dependent absorption, quadratic in the input intensity, is obtained due to two photon absorption (Id.). Since Figure 7 shows that dye1 has no absorbance at 1060 nm, it is apparent that the nonlinear transmission shown in Figure 9 results from simultaneous two-photon absorption (Id.). The absorbance required by the observed transmission shown in Figure 9 cannot be due to single photon excitation or sequential two-photon excitation, because dye1 is not capable of being excited to any level (either a full absorbance level or an intermediate one) at an incident wavelength of over 700 nm (Id.).

Figure 10 of the present application shows the absorbance spectrogram of a "dye1" doped film used in accordance with the present application, where this dye has no absorbance when subjected to incident photons with a wavelength greater than 600 nm, most specifically the wavelength of the Nd:YAG laser at 1060 nm (Stegeman Declaration ¶9). This dye and methods for its preparation are described on page 95 of the present application in Example 6 and on pages 98-99 in Examples 12-14 of the present application (Id.). Figure 11B, as described in Example 15 (pages 99-102 of the present application), shows that when dye1 (i.e. trans-4[p-(N-ethyl-N-hydroxyethyl amino) tetrapheny borate) is subjected to a Nd:YAG laser of a wavelength of 1060 nm (i.e. 1.06 μm), a photon is emitted at 600 nm due to two photon influenced fluorescence (Id.). Since Figure 10 shows that dye1 has no absorbance at 1060 nm, it is apparent that the emission shown in Figure 11B results from simultaneous two-photon excitation (Id.). The absorbance required by fluorescence shown in Figure 11B cannot be due to single photon excitation or sequential two-photon excitation, because dye1 is not capable of being excited to any level (either a full absorbance level or an intermediate one) at an incident wavelength of over 600 nm (Id.).

Based on all the foregoing, it is apparent that the present application discloses, to one of ordinary skill in the art, the use of simultaneous two-photon excitation (Stegeman

Declaration ¶10). Accordingly, the non-enablement rejection based on the simultaneous two-photon excitation limitation should be withdrawn.

The rejection of claims 311-312 and 321-325 under 35 U.S.C. § 112 (1st para.), because there is no enablement for a method of medical treatment of a particular volume of tissue by introducing a photo-active molecular agent into the tissue where it accumulates and directing light to specific regions of interest, is respectfully traversed.

These limitations are fully set forth on pages 69 to 70 of the present application. In particular, this passage states:

> It is well known in non-linear optics that two-photon induced processes exhibit the so-called "power threshold behavior", which means that the efficiency of the two-photon process scales with the square of the light's power. For this reason, high power densities are needed to trigger two-photon processes. This gives rise to yet another important clinical benefit. When the laser beam is tightly focused on the irradiated area to produce the light-induced two photon effect, no photochemical action will occur before or after the focal point of the irradiating laser beam.
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> * * *
>
> Although the mechanism by which the method of the present invention works has not been established, it is believed that the dye, upon irradiation, absorbs energy and transfers it to the photosensitizer which then leads to the killing of the cell or virus. It is unclear whether the transfer of energy from the dye to the photosensitizer proceeds radiatively, non-radiatively, or otherwise.
>
> * * *
>
> In addition to in vitro applications, the method of the present invention can be employed for the in vivo destruction of cells of a mammal, such as cells of abnormal or undesirable tissue. Like conventional PDT, the method is particularly useful for the killing of cells which replicate at an abnormally high rates, such as those of tumors. However, unlike conventional PDT methods, which are limited to shallow tumors, such as those of the head and neck, the methods of the present invention can also be used to treat deep-seated tumors, such as those in the pancreas. The method is

also useful for the destruction of neoplastics, such as bronchial, cervical, esophageal, or colon cancers.

Since the subject matter of claim 311-312 and 321-325 is fully enabled by the present application, it is submitted that the rejection of these claims should be withdrawn.

In view of all the foregoing, it is submitted that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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